REMARKS

Following the interview on July 30, 2003, the Applicants have amended the claims of the instant patent application. The Substance of the interview is attached to the Response to the Office Action.

I. Rejections Under 37 CFR 1.75.

The claims 9 and 11 were mistakenly repeated in the previous version of the application. As all the claims have been deleted and replaced by new claims, the rejection does not affect the Response.

II. Objections to lack of Sequence listing.

The sequences of arbitrary primers MCG1 and Blue and of cloned DNA fragment (band N of fingerprint generated by Blue primer) will be provided in a Supplemental Response. The sequence of arbitrary primer F was disclosed in the Specification. In a revised application the sequence will be allocated an identifier. Primer F was an arbitrary primer used for the generation of the amplotype of metastatic colorectal cancer (Malkhosyan et al, 1998), but only represented a few fingerprint bands from chromosomes 17 and 18.

III. Objections to embedded hyperlink in the Specification.

The hyperlink will be deleted from the instant application.

IV. Remarks on the lack of "genomic damage fraction" in the Provisional application.

The Office Action remarked that

> "the instant application claims priority to provisional application 60/096,828, filed August 17, 1998. The '828 application, does not provide support for the recitation of "genomic damage fraction" nor of "determining the risk of recurrence of colorectal cancer", therefore, the effective filing date of claims 1-11 is that of the instant application, August 16, 1999. (It is noted that the office action mailed 12/20/2000 rejected claims 1-23 over Arribas et al under 35 USC 102(a), however, with regard to claims 1-11, the rejection was in error, as such claims should have been rejected under 35 USC 102(b). Therefore, the declaration filed 8/3/01, is not sufficient to overcome the rejection with respect to claims 1-11).

Applicants respectfully request the Office Action to reconsider the negative conclusion, in light of the following considerations:

While it is acknowledged that theProvisional application of August 1998 did not explicitly

describe the concept of "genomic damage fraction", which is integral part of former claims 1-11, this is not a critical disqualification to the Instant Application for two reasons. First, the GDF term is not critical for the embodiment of the Instant Application. And second, the concept was implicitly described in the Specification of the Provisional Application.

1. The concept of the "genomic damage fraction" is not essential for the invention, as it is an arbitrary nomenclature to convey the concept that some tumors have more altered bands and consequently more damage to their genome than other tumors. We could also have used the term "fractional copy number changes" or "genomic damage index" etc.

2. The Provisional Application contained in its Specifications all the information necessary and sufficient to derive the concept contained in the term "genomic damage fraction" or GDF. The amplotype of colon cancer represents the accumulative number of the fingerprint bands exhibiting alterations, which are used to derive the GDF. In other words, from the data of Figures 5 and 6 of the Provisional Application it is possible to infer the GDF for the tumors analyzed, because information for the number of altered bands of each individual tumor is available. This is the case because otherwise the amplotype could not have been generated. The GDF is an arbitrary value reflecting the proportion of bands with alterations out of the total number of fingerprint bands for each tumor, but the GDF is only useful when applied to a group of several tumors. This allows the segregation of the tumors into two groups: tumors with a GDF higher than average, and tumors with a GDF lower than average (i.e. some tumors have more and some tumors have less altered bands than the average number of altered bands of the total number of tumors analyzed). For instance, figure 5 of the Specification of the Instant Application shows that gains of bands from chromosome 6 or losses of bands from chromosome 4 occur in about 50% of the tumors. This means that about half of the 25 tumors examined in that particular experiment had gains or losses, but the other half did not. Thus, the 50% of the tumors with the alterations would have a higher specific GDF for the bands at chromosome 6 and 4 than the tumors without the alteration, and the same with the rest of the bands. Therefore, the availability of the information that yielded Figure 5 will automatically yield a GDF for the 25 tumors analyzed in that particular experiment. Precisely because of the availability of the information generating Figure 5, Applicants gave for granted that the Provisional Application automatically included the information for GDF and cancer prognosis.

The argument that the Provisional application did not contain a description supporting the recitation of "determining the risk of recurrence of colorectal cancer" is not critical to the Instant Application, as the revised claims do not include this terminology. Nevertheless, the risk of recurrence of colorectal cancer is implicit in the clinical outcome, for instance, the survival curve depicting the differential survival of colorectal cancers with or without the losses of band N of Blue primer (Figure 7 of Instant Application, and description in page 9). The survival curve of Figure 7 was derived by plotting the patients that died from colorectal cancer, therefore from recurrent colorectal cancer (in opposition to death from other causes), versus the patients that remained alive at the time of follow-up. The identification of loss of band N of Blue primer in a colorectal tumor from a given colorectal cancer patient thus has prognostic value to calculate the risk of this patient to have a recurrent colorectal cancer sometime in the future, and thus, an increased risk of dying from [recurrent] colorectal cancer.

Furthermore, the Provisional Application contains a description of the prognostic value of the altered fingerprint bands and the GDF in page 14: "It [AP-PCR DNA fingerprinting] also gives an overall picture of the *extent of genetic damage* in tumor cells which may have prognostic value for cancer (24)". The "extent of genetic damage" is functionally identical to the "genomic damage fraction". The revised claims of the instant application could be easily modified by substituting "genomic damage fraction" by "extent of genetic damage" without affecting its scope.

In conclusion, Applicants respectfully traverse the Office Action objection over *priority,* (pages 3 and 4) and the *Claims Rejections - 35 USC §102* (pages 9 and 10) in regards to the Arribas et al paper from 1997, for claims 1-11. The Provisional Application should thus be considered dominant regarding these date priority issues.

V. Rejections Under 35 U.S.C. § 112, first paragraph

The Office Action rejected several of the prior claims because they introduced new matter not recited in the Original Application or the Specifications. In particular, the Office Action stated

> Such amendments introduce new matter into the claims because the claims now encompass losses and gains of complete chromosomes, which was not recited in either the specification as filed, or the originally filed claims. Both the specification and claims recite that 'regions" of chromosomes 4 and 6 are lost and gained, respectively, and do not suggest the loss or gain of complete chromosomes.

The loss or the gain of an AP-PCR fingerprint band may be due to the loss or the gain of the entire chromosome, the entire chromosome arm, or only a portion of the chromosome arm where the band is located. The previously amended claim was not intended to specify "entire chromosome". The new claims do not include these chromosomes and the wording has been revised in Applicants' best attempt to avoid ambiguity.

VI. Rejections Under 35 U.S.C. § 112, second paragraph

Under this section, the Office Action rejected the claims, arguing the indefinite and ambiguous nature of the wording. The revised claims are rewritten in an attempt to avoid ambiguity. The concept of genomic damage has been restricted to the estimation achieved by AP-PCR fingerprinting; the clinical outcome has been narrowed to "death from recurrent colorectal cancer" or "absence of death from recurrent colorectal cancer"; "nucleic acids" have been replaced by the more restrictive and precise "genomic sequences"; and so on.

The invention rests on the value for cancer prognosis of alterations in the cancer cell genome as reflected in the alterations of the DNA fingerprint bands, and the fact that such an experimental approach is so simple in comparison with other methods. Applicants do not intend to overstate the claims or to unjustifiably attempt to widen and generalize them.

VI. Rejections Under 35 U.S.C. § 102

Under this section, the Office Action rejected the claims because of theArribas et al paper of 1997. The issue has been already addressed in the previous section IV.

The Office Action also rejected claim 21 under this 35 U.S.C. § 102 section as being anticipated by Peinado et al (Proceedings of the National Academy of Sciences, USA. Vol. 89, pp 10065-10069; November 1992). The claim has been revised to avoid the rejection.

VI. Rejections Under 35 U.S.C. § 103

Under this section related to the obviousness of the invention because of prior art, the Office Action remarked the issue of the timing of the individual inventor in the process that lead to the different claims of the instant application. The first Applicant, M.P., was involved in the invention from its inception to its last stages. Thesecond Applicant, S.M., was responsible for the work that originates the invention under the overall direction of the first Applicant (Malkhosyan et al, Proceedings of the Natl. Academy of Sciences, 95, 10170-1075, 1998). In the GDF claims (claims 1-11) the second Applicant also contributed intellectual input.

13. Claims 13-20 were rejected under 35 U.S.C. 103(a) as being unpatentable over Perucho (Genomic Instability and Carcinogenesis, pp 42-45, 1996) in view of Yasuda et al (Genomics, vol. 34, pp 1-8, 1996) (hereinafter referred to as Yasuda).

Applicants respectfully traverse the rejection. There is no disclosure in Perucho 1996 in view of Yasuda 1996, because the current invention rests on the useful application for cancer prognosis of the alterations identified by AP-PCR. There is no description of prognostic value of these alterations in either Perucho 1996 or Yasuda 1996. The Office action stressed the fact that "Perucho [1996] teaches that using this method, a molecular karyotype (or amplotype) of *metastatic* colon cancer was generated, ...". The fact that the amplotype of metastatic tumors was generated does not imply any obvious value for prognosis, as the prognostic value is always a relative estimation by comparing patients with poor or good survival. The generation of an amplotype for metastatic [colorectal] cancer does not provide *per se* any insight as to clinical outcome as all the tumors analyzed were at the same stage of progression. Only the comparison of the relative extent of genomic damage (i.e. GDF) or the identification of particular altered bands may have prognostic value for cancer survival and there is no disclosure of this in either Perucho 1996 or Yasuda 1996 (see also attached Substance of Interview of July 30, 2003).

14. Claim 23 is rejected under 35 U.S.C. 103(a) as being unpatentable over Peinado et al in view of Yasuda et al (Genomics, vol. 34, pp 1-8, 1996) (hereinafter referred to as Yasuda).

Applicants respectfully traverse the rejection. There is no disclosure in Peinado et al. 1992 in view of Yasuda 1996, because the current invention rests on the useful application for cancer prognosis of the alterations identified by AP-PCR. There is no information in Peinado et al. 1992 that could make obvious the experimental strategy that forms the body of the instant application. Peinado 1992 teaches that the colorectal cancers analyzed included metastatic carcinomas, but in no instance the analysis included the primary and metastatic carcinomas from the same patients, and in any case Peinado 1992 teaches the casual link between altered AP-PCR bands and cancer prognosis. The Office Action conclusion that

> Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the blue primer as taught by Yasuda, in the method of Peinado, for the obvious improvement of obtaining a representation of as many chromosomes as possible in the method of Peinado (it is noted that Peinado is silent as to which chromosomes are represented by the primers used in the method of Peinado). The ordinary artisan would have been motivated to use the blue primer of Yasuda to obtain chromosomal representations that would not be possible with the MCG1 primer of Peinado,

> and would have recognized that the method ofPeinado would be more complete if as many chromosomes as possible were represented in the method of Peinado to detect chromosomal regions of gains and loss associated with colorectal cancer.

is therefore respectfully traversed in regards to the obviousness of the application of the invention for cancer prognosis.

However, the Office Action's conclusion is wholeheartedly agreed upon by Applicants as it can serve to illustrate the generalization of the invention to any cancer. The current invention rests on the applicability and usefulness for cancer prognosis of the alterations observed in the AP-PCR fingerprints of tumor versus normal tissues of the same cancer patients. The Instant Application provided examples of such value using colorectal cancer. But the methods exemplified for colorectal cancer can be applied to any cancer as taught.

The first level of usefulness is the estimation of the overall extent of genomic damage (GDF) that can be utilized in a prospective manner once the gold standard for GDF is determined, for instance, by using a determined set of arbitrary primers, such as MCG1 and Blue. Any isolated tumor surgically removed will yield a GDF by performing two simple AP-PCR experiments comprising the amplification of four PCR reactions in only four microtubes: normal and tumor DNA with MCG1primer and normal and tumor DNA with Blue primer. The information obtained in this simple manner will be sufficient to predict clinical outcome of the given patient, by determining whether the GDF is lower or higher than the gold standard average GDF previously determined in the same experimental conditions for a group of tumors with known clinical outcome. The same simple principle can be applied to any other malignancy, as long as the overall GDF has been determined for a group of tumors of the same class. During the interview on July 30, 2003, the Applicant provided data demonstrating the generalization of the invention for gastric cancer. The manuscript reporting the prognostic value of the GDF derived by AP-PCR of two primers, MCG1 and Blue will be provided in a Supplemental Response. While the invention has not been carried out to practice to other malignancies, the mechanisms underlying the experimental strategy are universal and the prediction can be made that, as the Office Action states, it would be prima facie obvious to one of ordinary skill in the art at the time the invention to apply the same principle and the same simple methodology to any other malignancy with the same predictable result: the higher the GDF, the worse the clinical outcome. The generalization of the invention is also supported by the previous broad claim granted to a prior similar invention generating a similar value for a partial estimation of genomic damage, the fractional allelic loss or FAL (US Patent 5,580,729, generalized

method for cancer assessment. B. Vogelstein. December 3, 1996). The instant application represents a considerable improvement over that previous invention for its superior simplicity.

The second level of usefulness of the invention relates to the prognostic value of any individual fingerprint band as taught for band N of Blue primer. The same experiment that will yield the GDF for the particular patient will also provide information for the status in the tumor cell genome of the particular DNA sequences that comprises band N of Blue primer. The information also has immediate prognostic value, as it was shown that loss of this band correlated with poor survival. Therefore, the identification of the status of such individual band (loss or no loss) will predict the risk of death from recurrent (metastatic) cancer. Once the link between altered band and survival has been obtained for a given type of tumor, as taught for the band N of Blue primer for colorectal cancer, the determination of the band status will be also similarly useful for prognosis of that particular cancer. It is obvious, that there is no possible prediction of the value of any given band for any given type of cancer, *a priori* as this will need to be experimentally established. But the prediction can be made that given a sufficient number of fingerprint bands analyzed (such as the bands obtained with two arbitrary primers, MCG1 and Blue) there will be some bands that will have predictive and prognostic value for that particular type of cancer. Evidence supporting the prediction was also provided during the Interview on July 30, 2003 for another band amplified by Blue primer, band G3, also located in chromosome 4, whose allelic imbalance (loss of one allele and gain of the other) was shown to be of prognostic value for gastric cancer. Several additional examples of prognosis-valuable bands are currently available and will be provided in a Supplemental Response. Furthermore, similar findings were made with a similar study involving breast cancer. This time, the useful band was a band located in chromosome X. A publication reporting this finding was provided during the Interview on July 30, 2003 and will be also provided in a Supplemental Response. Therefore, it is possible to generalize the success of the invention in finding DNA fragments whose losses or gains provide prognostic markers for cancer. Due to the intrinsic peculiarity of the AP-PCR DNA fingerprinting method, that uses primers of arbitrary sequence, the following hypothetical experiment can be described:

Take a dice with four sides labeled A, C, G and T. Throw the dice to get a random sequence of 20 bases. Write down the sequence. Order a primer with that sequence (this was the procedure utilized for determining the nucleotide sequence of the Blue primer) and use it for generating DNA fingerprints of ~100 matched cases of normal-tumor DNAs from lung, or prostate, or any other

malignancy, whose clinical outcome is known (i.e., who died and who survived). Score the fingerprint bands with alterations. Search for correlation of the altered band with poor (or good) outcome. Select the band(s) with prognostic value. Apply the DNA fingerprinting to new individual cases of the same type of cancer. Determine the status of such band(s) (loss or no loss, or gain or no gain) by a simple two tube PCR amplification in AP-PCR experimental conditions, and predict outcome of said cancer patient: relative risk of dying from recurrent cancer (i.e., less than 20 probability of cancer free survival in 5 years; more than 80% chance of cancer free-survival in the next 5-year period, etc).

For such a straightforward approach, there is no need to know anything previously, other than the clinical outcome of a panel of cancer patients, and the availability of tissue specimens from the cancer patients. No information is necessary for the nature of the DNA fragment that composes the prognostic valuable fingerprint band(s), nor their chromosomal localization. However, it is also obvious that the availability of a database of AP-PCR arbitrary primers with the corresponding AP-PCR fingerprint bands with known chromosomal localization can facilitate the application of the invention.

In conclusion, pursuant to the above amendments and remarks, reconsideration and allowance of the revised claims in this application is believed warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313-1450, on the date shown below.

Manuel Perucho — August 13, 2003.
Date